Application note

Solid-state culture of thermophiles in the Terrafors-IS

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1. Introduction

The use of solid-state fermenters (SSF) has long been a specialised area of biotechnology, which is now receiving more attention, due to the rise of bioremediation applications and enhanced production of enzymes on solid substrates. Often, these fermentations require harsher environmental conditions and/or a relatively long time for a suitable microbial population to become established.

A number of designs of solid-state bioreactors exist but the challenge is to create equipment which has the type of versatility associated with the stirred tank reactor (STR). The Terrafors-IS provides just such a solution with in-situ sterilisation, process control and simple operation.

This application note describes a simple cultivation protocol for thermophilic microbes in the Terrafors-IS.

2. Technical specifications of the Terrafors-IS

- Vessel: 15 L total volume (up to 4 kg solids or 7 L liquids/slurries)
- Gassing with air, manual control via rotameter – mass flow valve optional
- Exit gas cooler as standard to minimise evaporation losses
- Mixing by rotation, clockwise or anticlockwise.
- Temperature control with electrical heating and cooling valve
- In-situ sterilisation, via a double jacket and direct steam injection
- Data logging of on-line parameters with Iris Parallel Bioprocess Control Software
- Sampling as standard

3. Experimental conditions

a) Substrate

Commercial **bran** was used for all experiments.

b) Parameter settings for the Terrafors-IS

Sterilisation:

- Temperature of sterilisation: 115°C
- Duration of sterilisation: 15 min
- V808 steam injection valve pulsing: 50%
- Speed: 5 rpm

Operation:

- Duration: 598 hours (25 days)
- Temperature of fermentation: 45°C
- Speed: starting with 0 rpm, then intermittent mixing (-5 to 5 rpm)
- Flow: 0.5 L/min

4. Solid-state culture

The chamber was loaded with 1.785 kg of substrate and sterilised in accordance with the parameters shown above. Determination of the moisture content after sterilisation, required double sterilisation only for that part of the experiment.

Inoculation was made by adding 2 strips of agar containing fungi of strains **TF-84 and TF-85.** The substrate and inoculum were then mixed by rotating the chamber at 5 rpm clockwise (+5 rpm) and anticlockwise (-5 rpm) at 10 minute intervals for 1 hour. Mixing was then set to zero.

Fig. 1: INFORS HT Terrafors-IS

After 16 hours the CO_2 concentration in the exit gas stream was measured using an external gas analyser and recorded using the Iris Parallel Bioprocess Control Software. The inoculum mixing strategy was modified by setting -5 rpm for 5 minutes, 1 minute's pause, then 5 rpm for 5 minutes before stopping the stirrer and waiting 1 hour before starting the cycle again. On subsequent days, a variety of alternative mixing speeds and durations were tried within the parameter given (see figures 2 and 3 below).

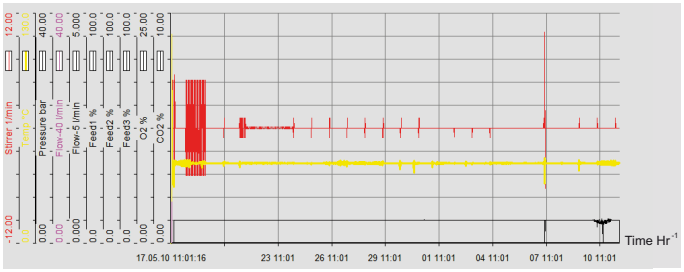

Fig. 2: Terrafors-IS fermentation at 45°C for 25 days

5. Analysis

Moisture content of the substrate

The purpose of the test was to quantify the moisture added to the substrate at the time of its sterilisation (by determining the dry weight after 48 hours out of drying oven set to 50°C with an initial mass of 150 gram).

At the end of the sterilisation, 307.1 g of wet wheat bran was taken for the determination of the dry weight (68.97% of final moisture instead of the theoretical 66.53% calculated).

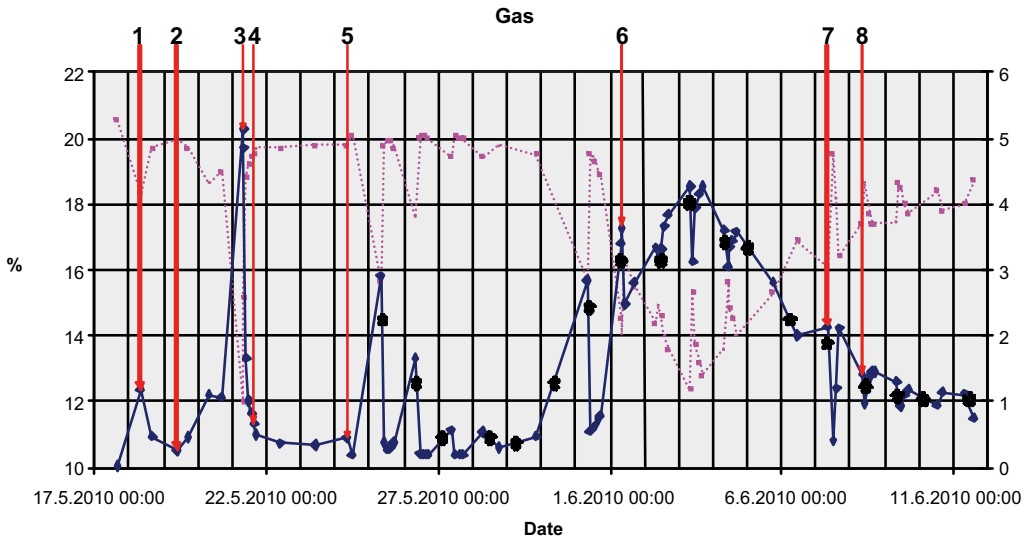

Fig. 3: Exit gas analysis for O_2 and CO_2 for different mixing strategies

Start point = stirrer: 0 rpm
1 = stirrer: -5 rpm for 5 minutes, 1 minute pause, 5 rpm for 5 minutes, 1 hour pause (then repeated)
2 = stirrer: 0 rpm
3 = stirrer: -1 rpm for 1 hour (then repeated)
4 = stirrer: -0.1 rpm for 1 hour, 0.1 rpm for 1 hour (then started again)
5 = stirrer: -1 rpm for 30 minutes, 1 minute pause, 1 rpm for 30 minutes, 22:59 pause (then repeated)
6 = stirrer: -1 rpm for 1 minute, 1 minute pause, 1 rpm for 1 minute, 23:57 pause (then repeated)
7 = stirrer: 0 rpm (sampling and addition of TB-154)
8 = stirrer: -1 rpm for 1 minute, 1 minute pause, 1 rpm for 1 minute, 23:57 pause (then repeated)

6. Results and evaluation

Sterilisation is a critical step, and much experimental work (unpublished data) has been performed to determine the optima for steam injection and gas flow rate for good growth of the inoculum. Water of activation is an important factor. And the best value is found around the naturally occuring maximum for a given substrate. Moisture content can be controlled by the amount of steam injected into the inner chamber during sterilisation with subsequent gas flow rate playing a lesser role.

Excessive moisture causes more of the substrate to "cake" onto the walls of the inner chamber, so careful control is necessary for this reason also.

During the fermentation, it was noticeable that the fungal culture was very sensitive to the adjustment of the stirrer. Its activation instantly made the measured value of exit CO_2 fall i.e. the more they are disturbed the less well they perform, due to physical breakdown of the mycelium network in the substrate. The optimum adjustment of the stirrer was estimated to be 1 rpm for 1 minute, 1 minute of pause, -1 rpm for 1 minute, followed by a pause of 23 hours and 57 minutes.

Parameter data

The initial in-situ sterilisation is an important step in optimising the moisture content of the substrate. This proves to be equivalent to the natural maximum for water content of the chosen substrate.

The plot shown in figure 2 shows that the parameters were maintained close to optimum for the entire batch culture. Temperature reached set point quickly and then was maintained.

Regulation of mixing was critical to productive growth, as measured by the value for CO_2 in the exit gas stream. The level of carbon dioxide was measured using an infrared CO_2 detector (INFORS HT).

Figure 3 shows the nature of this relationship. The objective was to determine a mixing strategy which optimised the growth of the fungal inoculum and this has been found i.e. mixing for 1 minute in each direction with a one minute pause between every 24 hours.

7. Summary

- Cultivation of mixed thermophilic fungi in batch mode was achieved using a readily available solid-state substrate in the Terrafors-IS.
- Sterilisation was effective in the Terrafors-IS, even at temperatures as low as 115°C.
- The amount of steam injected into the inner chamber is controlled via a pulse valve (10–100 % opening time) and this value is critical for optimising the moisture content of the substrate.
- These optima were shown to be close to the natural maximum water content of the substrate.
- Gas flow rates had some effect on the moisture content post-sterilisation but this was not significant as a proportion of the total.
- Metabolic activity could be monitored using an exit gas analyser to detect the level of CO_2 in the exit gas stream.
- Mixing time and the duration of pauses had a critical effect on the CO_2 values. An optimum mixing strategy was found.

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